Analysis of bacterial adhesion forces

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Chapter 1

General Introduction

Bacteria adhere to virtually all natural and man-made surfaces. Initiated by their adhesion, micro-organisms grow on a surface and form complex multi-cellular structures, referred to as biofilms.[1,2] In biofilms, micro-organisms are protected against environmental attacks,[3–5] such as antimicrobials, and therefore biofilms are often the cause of persistent contamination or infection.[6–8] Studies on screening of different preventive measures and removal of biofilms[9] are gaining significant attention in various fields of application, ranging from food and water processing[10] to modern medicine[11] and dentistry (Fig. 1).[12]

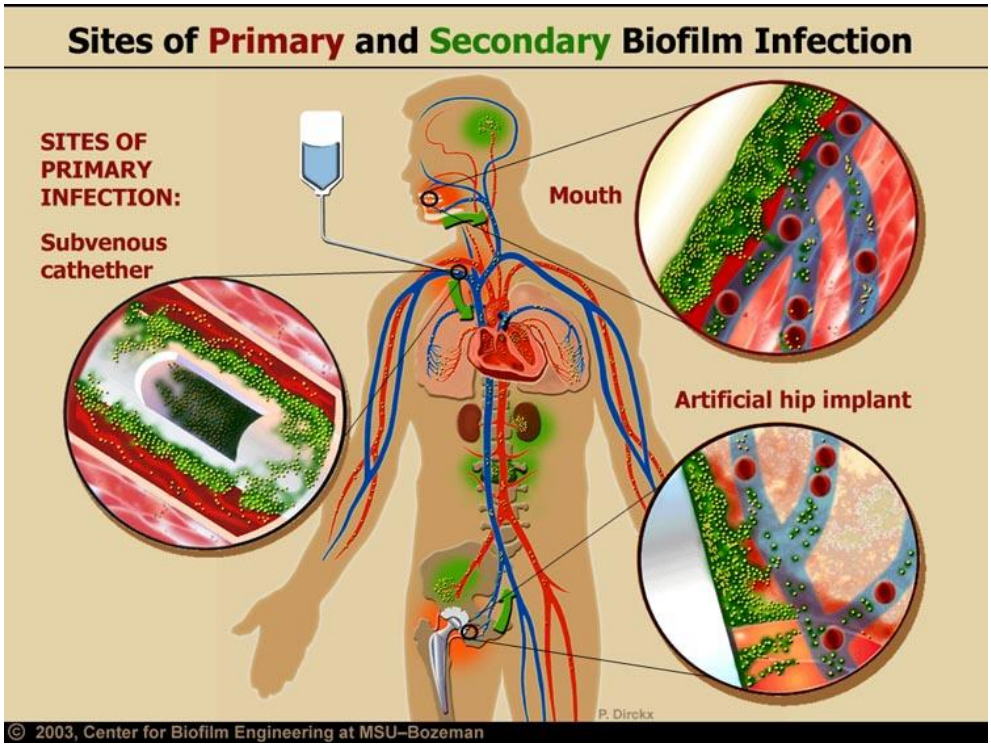

Figure 1. Common sites of primary and secondary (highlighted in green) biofilm infection in the human body. Source: Center for Biofilm Engineering, Montana State University-Bozeman.

Surface modification can be effective in reducing microbial adhesion to substratum surfaces,[13] while, interestingly, the susceptibility of a biofilm for antimicrobials in solution varies depending on the properties of the substratum surface. Different classes of antimicrobials, such as quaternary ammonium compounds or antimicrobial peptides, remain antimicrobially active when immobilized to a substratum surface.[14,15] Hypothetically, different generic mechanisms of action must exist for antimicrobials in solution and immobilized on a surface.

Insight into mechanisms of biofilm formation can lead to improved preventive mechanisms and requires knowledge of initial bacterial adhesion and the surface properties of the adhering bacteria.

BACTERIAL RESPONSE TO THEIR ADHESION ON A SUBSTRATUM SURFACE

It has been recently hypothesized[16] that an adhering bacterium responds to a substratum surface depending on the strength of the force by which it adheres. In this respect, three regimes of adhesion forces have been proposed (Fig. 2):

1. A “planktonic” regime, where the adhesion force is very weak so that the bacteria hardly sense that they are on a surface and therefore remain in their planktonic state,[17–19] in which they are susceptible to antimicrobials;

2. An “interaction” regime, where adhesion forces induce a cascade of genotypic and phenotypic changes[20] that render the organism more resistant to antimicrobial agents;[21,22]

3. A “lethal regime” in which strong adhesion forces de-activate adhering bacteria to impede growth and cause cell death.[16,23]

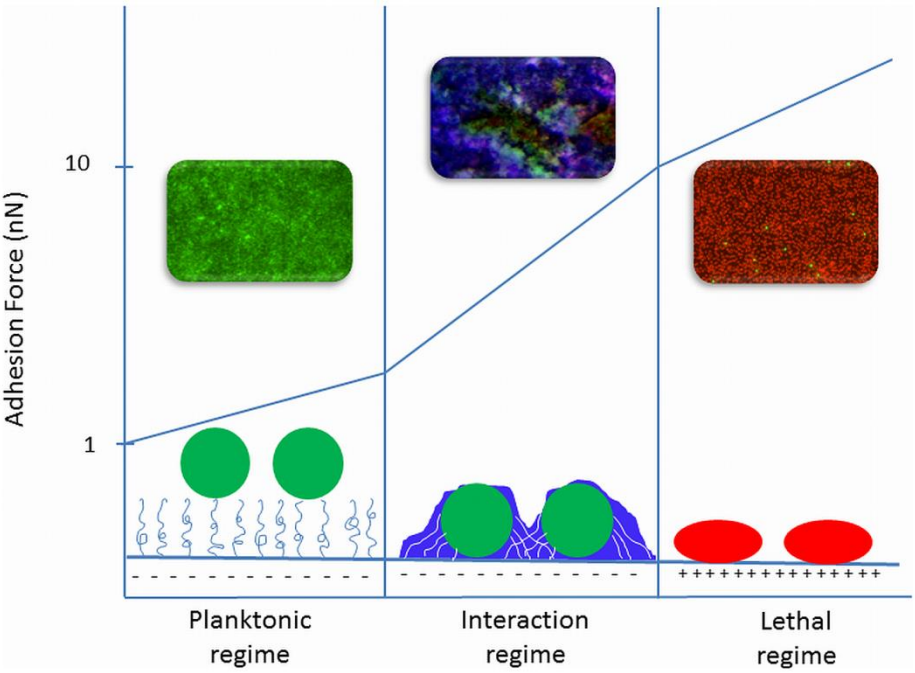

Figure 2. Three regimes of bacterial adhesion forces to substratum surfaces that dictate the bacterial response to a surface. (Illustration adapted from Busscher and Van der Mei[16])

In the different regimes of adhesion forces, it can be envisaged that a bacterium will undergo different degrees of cell wall deformation to which the organism subsequently responds. The term "stress-deactivation" has been coined for this hypothetical phenomenon.[23] Accordingly, knowledge of the forces by which bacteria adhere to a surface are of pivotal importance in understanding further events during biofilm formation.

BACTERIAL ADHESION FORCES USING ATOMIC FORCE MICROSCOPY (AFM)

Bacterial adhesion to surfaces can be assessed using various biochemical and physico-chemical approaches.[24,25] Among these methods, AFM, right after its invention in the 1970s,[26,27] showed most promising.[28,29] The distance dependence

of the interaction force between a bacterium and a substratum surface can be recorded using AFM as a force-distance curve with an approach and retract stage (Fig. 3). The magnitude of the adhesion force is usually determined by the rupture force when detaching the AFM cantilever, equipped with a bacterial probe, from the substratum surface.

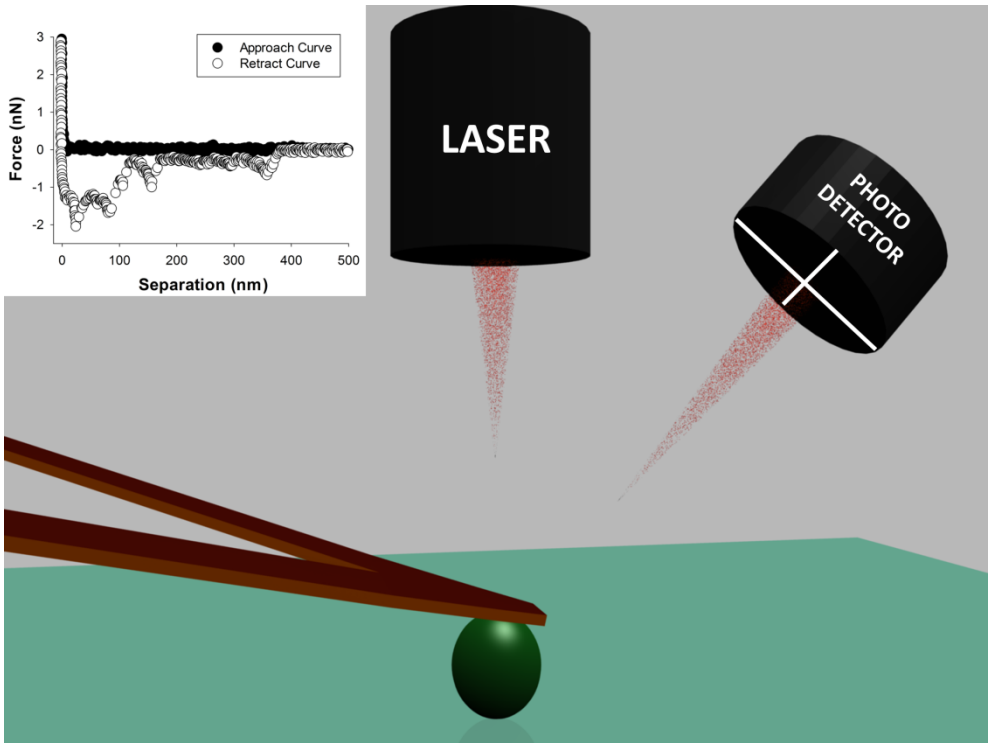

Figure 3. Illustration of measuring bacterial interaction forces using AFM and an example of the approach and rectract force-distance curves.

Theoretical models based on the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory[30,31] and its extended version (the X-DLVO theory)[32] are widely applied to derive the long-range (*e.g.* Lifshitz-Van der Waals and electrical double-layer interactions) and short-range (*e.g.* Lewis acid-base interactions) contributions to the total adhesion force, based on measured zeta potentials and contact angles

using various liquids.[32] The statistics-based Poisson analysis of bacterial adhesion forces measured using AFM provides a more experimental alternative to distinguish between long-range and short-range contributions to the overall interaction force.[33,34] However, these methods do not always concur in the magnitude and even the attractive or repulsive nature of the short-range interaction.[35,36] It has been argued that (X)DLVO-based models fail to take microscopic bacterial cell wall structures into consideration, whereas Poisson analysis of AFM-based adhesion forces includes effects of the microscopic feautures of the interface between a bacterium and a substratum surface.

AIM OF THIS STUDY

The aim of this thesis is to determine bacterial adhesion forces to substratum surfaces and associated cell wall deformation and evaluate their role in bacterial susceptibility to antimicrobials, either in solution or immobilized to a substratum surface.

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